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ENZYME NITRATE REDUCTASE OF SOME PARASITIC FUNGI

by

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ABSTRACT

Eight parasitic fungi, *Fusarium avenaceum*, *F. oxysporum*, *Gliocladium roseum*, *Phytophthora cinnamomi*, *Pythium debaryanum*, *Poria weirii*, *Trichothecium roseum*, and *Verticillium alboatrum*, were grown in liquid culture to determine the ability of each to produce nitrate reductase, an enzyme necessary to convert nitrate nitrogen into the more easily used ammonium form.

Cell-free enzyme preparations from the eight fungi (and from *Neurospora crassa* as a check) were made after 2 weeks' growth and assayed for nitrate reductase activity. Apparently all fungi tested except *Poria weirii* produced the enzyme.

Inability of *Poria weirii* to produce nitrate reductase could have important implications in the biological control of this important forest root pathogen.

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Many microorganisms use nitrate as their major source of nitrogen, converting it to ammonium forms or amino acids for protein synthesis. Some anaerobic organisms, moreover, employ nitrate as a terminal electron acceptor in place of oxygen, an energy-yielding reaction ("nitrate respiration") necessary for growth. The enzyme nitrate reductase, which is required as a catalyst for nitrate reduction, was originally partially purified and characterized from *Neurospora crassa* Shear and Dodge.[2] In an earlier study,[3] we found that *Poria weirii* Murr., a common root pathogen of many western conifers, lacks nitrate reductase and cannot assimilate nitrate. Since then, we have examined additional, hitherto untested, fungal parasites for nitrate reductase.

These fungi were obtained from the American Type Culture Collection:

Fusarium avenaceum	8150
Fusarium oxysporum	659
Gliocladium roseum	10521
Phytophthora cinnamomi	10922
Pythium debaryanum	9998
Trichothecium roseum	12519
Verticillium alboatrum	13642

We also retested *Poria weirii*, using the same isolate as before, because our technique had been modified for greater sensitivity in detecting nitrate reductase. *Neurospora crassa* was used as a check organism.

The isolate of each species was grown aerobically in three replicate 500-milliliter flasks containing 250 milliliters of synthetic medium on a reciprocating shaker in darkness at 24° C. The medium developed by Trione[4] was used, except that 10 grams glucose were included as a carbon source rather than 20 grams sucrose, and 0.1 percent potassium nitrate was added to the medium. L-asparagine was omitted for all but *Poria weirii*, which did not grow with KNO_3 as the only nitrogen source.

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[2] Nason, A., and Evans, H. J. Triphosphopyridine nucleotide nitrate reductase in *Neurospora*. J. Biol. Chem. 202: 655-673. 1953.

[3] Li, C. Y., Lu, K. C., Trappe, J. M., and Bollen, W. B. Selective nitrogen assimilation by *Poria weirii*. Nature 213: 814. 1967.

[4] Trione, E. J. Isolation and *in vitro* culture of the wheat bunt fungi *Tilletia caries* and *T. controversa*. Phytopathology 54: 592-596. 1964.

After 2 weeks, mycelia of two replicate flasks were collected in a Buchner funnel, washed with ice-cold, glass-distilled water, and ground with 90-mesh alundum powder for 30 minutes in a cold mortar. The resulting material was ground for 10 minutes with five times the cell weight of cold 0.1 molar phosphate buffer (pH 7.1), then centrifuged at 0° C. for 20 minutes at 2,000 gravity. The sediment was discarded and the supernatant recentrifuged at 0° C. for 40 minutes at 20,000 gravity. The cell-free extract segregated by the second centrifuging was used in determination of enzyme activity. The remaining cultures were harvested at the same time; the mycelia were dried at 85° C. for 1 day and weighed. These dry weights provided a basis for expressing enzyme activity.

Enzyme activity was determined by the procedure of Egami and Taniguchi.[5] One milliliter of cell-free extract was incubated anaerobically for 8 hours at 27° C. in mixture with 0.5 milliliter flavin adenine dinucleotide-formate and 2.0 milliliter of 0.01 molar KNO_3 (the 2-hour incubation period used by Egami and Taniguchi proved inadequate for the organisms we studied). Colorimetric determination of nitrite formed during incubation, a reaction requiring nitrate reductase as a catalyst, then provided an index of nitrate reductase present in the cell-free extract. The activity was expressed as µ moles of nitrite per milligram of dry mycelium.

This method for determining nitrate reductase activity uses nitrate as a hydrogen acceptor, the nitrate serving as a terminal oxidant for a reduced intermediate. Because inorganic nitrate provided the sole nitrogen source for all cultures except *P. weirii*, positive results indicate functioning systems for nitrate assimilation.

Of the fungi tested, all but *P. weirii* produced nitrate reductase (table 1). Organisms must produce nitrate reductase to assimilate nitrate nitrogen. Hypothetically, relative efficiency in synthesizing this enzyme influences an organism's ability to compete for available nitrogen. If true, this hypothesis has important implications for biological control of pathogens in soil through control of kinds and amounts of available nitrogen.

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[5] Egami, F., and Taniguchi, S. Nitrate. *In* Methods of enzymatic analysis. Ed. by Hans-Ulrich Bergmeyer. Pp. 636-639. New York: Academic Press. 1963.

Table 1.--*Nitrate reductase activity of cell-free extracts from two replicate cultures each of selected fungi, expressed as μ moles of nitrite formed per milligram of dry mycelium after incubation of crude enzyme extract with 2 milliliters of 0.01 molar* KNO_3 *and 0.5 milliliter FAD-formate solution*

Fungus	Nitrite formed	
	Per replicate	Mean
	- - μ moles x 10^{-2} per mg. mycelium - -	
Fusarium avenaceum	0.547	0.556
	.564	
Fusarium oxysporum	.215	.220
	.224	
Gliocladium roseum	8.110	8.110
	8.110	
Neurospora crassa	4.600	4.600
	4.600	
Phytophthora cinnamomi	.476	.503
	.529	
Pythium devaryanum	1.210	1.210
	1.210	
Poria weirii	0	0
	0	
Trichothecium roseum	.632	.551
	.469	
Verticillium alboatrum	.155	.155
	.155	